HPV 58 L1 Nucleotide Sequence Alignment

```
58 L1 wt  (   1)  ATGTCCGTGTGGCGGCCTAGTGAGGCCACTGTGTACCTGCCTCCTGTGCC
58 L1 R   (   1)  ........C...A.A..ATCC..A..T..C..C...T....A..A..T..

58 L1 wt  (  51)  TGTGTCTAAGGTTGTAAGCACTGATGAATATGTGTCACGCACAAGCATTT
58 L1 R   (  51)  A..C..C.....C..CTC......C.....C..C..TA.A..CTCT..C.

58 L1 wt  ( 101)  ATTATTATGCTGGCAGTTCCAGACTTTTGGCTGTTGGCAATCCATATTTT
58 L1 R   ( 101)  .C..C..C.....TTCC..T...T.G..........T..C.....C..C

58 L1 wt  ( 151)  TCCATCAAAAGTCCCAATAACAATAAAAAAGTATTAGTTCCCAAGGTATC
58 L1 R   ( 151)  ........GTC...A..C.....C..G..G..C..G.....A.....C..

58 L1 wt  ( 201)  AGGCTTACAGTATAGGGTCTTTAGGGTGCGTTTACCTGATCCCAATAAAT
58 L1 R   ( 201)  T..T..G..A..C..A.....C..A..CA.A..G..A..C..A..C..G.

58 L1 wt  ( 251)  TTGGTTTTCCTGATACATCTTTTTATAACCCTGATACACAACGTTTGGTC
58 L1 R   ( 251)  .C.....C..A..C..T..C..C..C.....A..C..T...A.A......

58 L1 wt  ( 301)  TGGGCATGTGTAGGCCTTGAAATAGGTAGGGGACAGCCATTGGGTGTTGG
58 L1 R   ( 301)  .....T.....C..TT.G.....C.....A..T..A.............

58 L1 wt  ( 351)  CGTAAGTGGTCATCCTTATTTCAATAAATTTGATGACACTGAAACCAGTA
58 L1 R   ( 351)  T..CTC......C..A..C.....C..G..C..C.....C......TCC.

58 L1 wt  ( 401)  ACAGATATCCCGCACAGCCAGGGTCTGATAACAGGGAATGCTTATCTATG
58 L1 R   ( 401)  .......C..A..T..A.....T.....C.....A.....T..G..C...

58 L1 wt  ( 451)  GATTATAAACAAACACAATTATGTTTAATTGGCTGTAAACCTCCCACTGG
58 L1 R   ( 451)  ..C..C..G.....C.....G.....G..C..T.....G..A..A.....

58 L1 wt  ( 501)  TGAGCATTGGGGTAAAGGTGTTGCCTGTAACAATAATGCAGCTGCTACTG
58 L1 R   ( 501)  ...A..C........G........T........C..C..T........C.

58 L1 wt  ( 551)  ATTGTCCTCCATTGGAACTTTTTAATTCTATTATTGAGGATGGTGACATG
58 L1 R   ( 551)  .C.....A.........T.G..C..C..C..C..C..A..C.........

58 L1 wt  ( 601)  GTAGATACAGGGTTTGGATGCATGGACTTTGGTACATTGCAGGCTAATAA
58 L1 R   ( 601)  ..C..C..T..T..C..T..T........C.....C.....A.....C..
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FIG. 1A

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58 L1 wt  ( 651)  AAGTGATGTGCCTATTGATATTTGTAACAGTACATGCAAATATCCAGATT
58 L1 R   ( 651)  GTCC..C..T..A..C..C..C......TCC..C..T..G..C.....C.

58 L1 wt  ( 701)  ATTTAAAAATGGCCAGTGAACCTTATGGGGATAGTTTGTTCTTTTTTCTT
58 L1 R   ( 701)  .C..G..G.....TTC......A..C..T..CTCC........C..CT.G

58 L1 wt  ( 751)  AGACGTGAGCAGATGTTTGTTAGGCACTTTTTTAATAGGGCCGGAAAACT
58 L1 R   ( 751)  ...A.A..A..A.....C..C..A.....C..C..C..A..T..T..GT.

58 L1 wt  ( 801)  TGGCGAGGCTGTCCCGGATGACCTTTATATTAAAGGGTCCGGTAATACTG
58 L1 R   ( 801)  G..T..A.....T..A..C...T.G..C..C..G..T..T.....C..C.

58 L1 wt  ( 851)  CAGTTATCCAAAGTAGTGCATTTTTTCCAACTCCTAGTGGCTCTATGGTT
58 L1 R   ( 851)  .T..C......TCCTC...T..C..C........ATC...T..C.....C

58 L1 wt  ( 901)  ACCTCAGAATCACAATTATTTAATAAGCCTTATTGGCTACAGCGTGCACA
58 L1 R   ( 901)  .....T.....T.....G..C..C.....A..C...T.G..AA.A..T..

58 L1 wt  ( 951)  AGGTCATAACAATGGCATTTGCTGGGGCAATCAGTTATTTGTTACCGTAG
58 L1 R   ( 951)  ......C.....C..T..C..T.....T..C..A..G..C..C..T..C.

58 L1 wt  (1001)  TTGATACCACTCGTAGCACTAATATGACATTATGCACTGAAGTAACTAAG
58 L1 R   (1001)  .C..C......A.ATC......C.....C..G..T..C.....C..C...

58 L1 wt  (1051)  GAAGGTACATATAAAAATGATAATTTTAAGGAATATGTACGTCATGTTGA
58 L1 R   (1051)  ........C..C..G..C..C..C..C........C..CA.A..C..C..

58 L1 wt  (1101)  AGAATATGACTTACAGTTTGTTTTTCAGCTTTGCAAAATTACACTAACTG
58 L1 R   (1101)  G.....C.....G..A..C..C..C..AT.G..T..G..C..CT.G....

58 L1 wt  (1151)  CAGAGATAATGACATATATACATACTATGGATTCCAATATTTTGGAGGAC
58 L1 R   (1151)  .T..A..C.....C..C..C..C..C.....C..T..C..C.....A...

58 L1 wt  (1201)  TGGCAATTTGGTTTAACACCTCCTCCGTCTGCCAGTTTACAGGACACATA
58 L1 R   (1201)  ........C.....G..T..A..A..A.....TTCC..G..A.....C..

58 L1 wt  (1251)  TAGATTTGTTACCTCCCAGGCTATTACTTGCCAAAAAACAGCACCCCCTA
58 L1 R   (1251)  C.....C..C.....T..A.....C..C..T.....G..T..T..A..A.
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FIG.1B

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58 L1 wt   (1301)   AAGAAAAGGAAGATCCATTAAATAAATATACTTTTTGGGAGGTTAACTTA
58 L1 R    (1301)   .G..........C.....G..C..G..C..C..C.....A..C.....G

58 L1 wt   (1351)   AAGGAAAAGTTTTCTGCAGATCTAGATCAGTTTCCTTTGGGACGAAAGTT
58 L1 R    (1351)   ..........C.....T..CT.G..C..A..C..A.....TA.......

58 L1 wt   (1401)   TTTATTACAATCAGGCCTTAAAGCAAAGCCCAGACTAAAACGTTCGGCCC
58 L1 R    (1401)   C..G..G.....T..TT.G..G..T.....A...T.G..GA.A..T..T.

58 L1 wt   (1451)   CTACTACCCGTGCACCATCCACCAAACGCAAAAAGGTTAAAAAATAA (SEQ ID NO:3
58 L1 R    (1451)   .A..C..TA.A..T..........GA.A..G.....C..G..G    (SEQ ID NO:1
```

FIG.1C

Synthetic HPV 58 L1 Nucleotide and Amino Acid Sequences.

```
        M  S  V  W   R  P  S   E  A  T   V  Y  L  P   P  V  P
    1   ATGTCCGTCT GGAGACCATC CGAAGCTACC GTCTACTTGC CACCAGTTCC
        TACAGGCAGA CCTCTGGTAG GCTTCGATGG CAGATGAACG GTGGTCAAGG
         V  S  K   V  V  S  T   D  E  Y   V  S  R   T  S  I  Y
   51   AGTCTCCAAG GTCGTCTCCA CTGACGAATA CGTCTCTAGA ACCTCTATCT
        TCAGAGGTTC CAGCAGAGGT GACTGCTTAT GCAGAGATCT TGGAGATAGA
          Y  Y  A   G  S  S   R  L  L  A   V  G  N   P  Y  F
  101   ACTACTACGC TGGTTCCTCT AGATTGTTGG CTGTTGGTAA CCCATACTTC
        TGATGATGCG ACCAAGGAGA TCTAACAACC GACAACCATT GGGTATGAAG
        S  I  K  S   P  N  N   N  K  K   V  L  V  P   K  V  S
  151   TCCATCAAGT CTCCAAACAA CAACAAGAAG GTCTTGGTTC CAAAGGTCTC
        AGGTAGTTCA GAGGTTTGTT GTTGTTCTTC CAGAACCAAG GTTTCCAGAG
         G  L  Q   Y  R  V  F   R  V  R   L  P  D   P  N  K  F
  201   TGGTTTGCAA TACAGAGTCT TCAGAGTCAG ATTGCCAGAC CCAAACAAGT
        ACCAAACGTT ATGTCTCAGA AGTCTCAGTC TAACGGTCTG GGTTTGTTCA
         G  F  P   D  T  S   F  Y  N  P   D  T  Q   R  L  V
  251   TCGGTTTCCC AGACACTTCC TTCTACAACC CAGACACTCA AAGATTGGTC
        AGCCAAAGGG TCTGTGAAGG AAGATGTTGG GTCTGTGAGT TTCTAACCAG
        W  A  C  V   G  L  E   I  G  R   G  Q  P  L   G  V  G
  301   TGGGCTTGTG TCGGTTTGGA AATCGGTAGA GGTCAACCAT TGGGTGTTGG
        ACCCGAACAC AGCCAAACCT TTAGCCATCT CCAGTTGGTA ACCCACAACC
         V  S  G   H  P  Y  F   N  K  F   D  D  T   E  T  S  N
  351   TGTCTCTGGT CACCCATACT TCAACAAGTT CGACGACACC GAAACCTCCA
        ACAGAGACCA GTGGGTATGA AGTTGTTCAA GCTGCTGTGG CTTTGGAGGT
         R  Y  P   A  Q  P   G  S  D  N   R  E  C   L  S  M
  401   ACAGATACCC AGCTCAACCA GGTTCTGACA ACAGAGAATG TTTGTCCATG
        TGTCTATGGG TCGAGTTGGT CCAAGACTGT TGTCTCTTAC AAACAGGTAC
        D  Y  K  Q   T  Q  L   C  L  I   G  C  K  P   P  T  G
  451   GACTACAAGC AAACCCAATT GTGTTTGATC GGTTGTAAGC CACCAACTGG
        CTGATGTTCG TTTGGGTTAA CACAAACTAG CCAACATTCG GTGGTTGACC
         E  H  W   G  K  G  V   A  C  N   N  N  A   A  A  T  D
  501   TGAACACTGG GGTAAGGGTG TTGCTTGTAA CAACAACGCT GCTGCTACCG
        ACTTGTGACC CCATTCCCAC AACGAACATT GTTGTTGCGA CGACGATGGC
         C  P  P   L  E  L   F  N  S  I   I  E  D   G  D  M
  551   ACTGTCCACC ATTGGAATTG TTCAACTCCA TCATCGAAGA CGGTGACATG
        TGACAGGTGG TAACCTTAAC AAGTTGAGGT AGTAGCTTCT GCCACTGTAC
        V  D  T  G   F  G  C   M  D  F   G  T  L  Q   A  N  K
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FIG.2A

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601   GTCGACACTG GTTTCGGTTG TATGGACTTC GGTACCTTGC AAGCTAACAA
      CAGCTGTGAC CAAAGCCAAC ATACCTGAAG CCATGGAACG TTCGATTGTT
       S  D  V   P  I  D  I   C  N  S   T  C  K   Y  P  D  Y
651   GTCCGACGTT CCAATCGACA TCTGTAACTC CACCTGTAAG TACCCAGACT
      CAGGCTGCAA GGTTAGCTGT AGACATTGAG GTGGACATTC ATGGGTCTGA
        L  K  M   A  S  E   P  Y  G  D   S  L  F   F  F  L
701   ACTTGAAGAT GGCTTCTGAA CCATACGGTG ACTCCTTGTT CTTCTTCTTG
      TGAACTTCTA CCGAAGACTT GGTATGCCAC TGAGGAACAA GAAGAAGAAC
      R  R  E  Q   M  F  V   R  H  F   F  N  R  A   G  K  L
751   AGAAGAGAAC AAATGTTCGT CAGACACTTC TTCAACAGAG CTGGTAAGTT
      TCTTCTCTTG TTTACAAGCA GTCTGTGAAG AAGTTGTCTC GACCATTCAA
       G  E  A   V  P  D  D   L  Y  I   K  G  S   G  N  T  A
801   GGGTGAAGCT GTTCCAGACG ACTTGTACAT CAAGGGTTCT GGTAACACCG
      CCCACTTCGA CAAGGTCTGC TGAACATGTA GTTCCCAAGA CCATTGTGGC
        V  I  Q   S  S  A   F  F  P  T   P  S  G   S  M  V
851   CTGTCATCCA ATCCTCTGCT TTCTTCCCAA CTCCATCTGG TTCCATGGTC
      GACAGTAGGT TAGGAGACGA AAGAAGGGTT GAGGTAGACC AAGGTACCAG
      T  S  E  S   Q  L  F   N  K  P   Y  W  L  Q   R  A  Q
901   ACCTCTGAAT CTCAATTGTT CAACAAGCCA TACTGGTTGC AAAGAGCTCA
      TGGAGACTTA GAGTTAACAA GTTGTTCGGT ATGACCAACG TTTCTCGAGT
       G  H  N   N  G  I  C   W  G  N   Q  L  F   V  T  V  V
951   AGGTCACAAC AACGGTATCT GTTGGGGTAA CCAATTGTTC GTCACTGTCG
      TCCAGTGTTG TTGCCATAGA CAACCCCATT GGTTAACAAG CAGTGACAGC
        D  T  T   R  S  T   N  M  T  L   C  T  E   V  T  K
1001  TCGACACCAC TAGATCCACT AACATGACCT TGTGTACCGA AGTCACCAAG
      AGCTGTGGTG ATCTAGGTGA TTGTACTGGA ACACATGGCT TCAGTGGTTC
      E  G  T  Y   K  N  D   N  F  K   E  Y  V  R   H  V  E
1051  GAAGGTACCT ACAAGAACGA CAACTTCAAG GAATACGTCA GACACGTCGA
      CTTCCATGGA TGTTCTTGCT GTTGAAGTTC CTTATGCAGT CTGTGCAGCT
       E  Y  D   L  Q  F  V   F  Q  L   C  K  I   T  L  T  A
1101  GGAATACGAC TTGCAATTCG TCTTCCAATT GTGTAAGATC ACCTTGACTG
      CCTTATGCTG AACGTTAAGC AGAAGGTTAA CACATTCTAG TGGAACTGAC
        E  I  M   T  Y  I   H  T  M  D   S  N  I   L  E  D
1151  CTGAAATCAT GACCTACATC CACACCATGG ACTCTAACAT CTTGGAAGAC
      GACTTTAGTA CTGGATGTAG GTGTGGTACC TGAGATTGTA GAACCTTCTG
      W  Q  F  G   L  T  P   P  P  S   A  S  L  Q   D  T  Y
1201  TGGCAATTCG GTTTGACTCC ACCACCATCT GCTTCCTTGC AAGACACCTA
      ACCGTTAAGC CAAACTGAGG TGGTGGTAGA CGAAGGAACG TTCTGTGGAT
       R  F  V   T  S  Q  A   I  T  C   Q  K  T   A  P  P  K
```

FIG.2B

```
1251    CAGATTCGTC ACCTCTCAAG CTATCACCTG TCAAAAGACT GCTCCACCAA
        GTCTAAGCAG TGGAGAGTTC GATAGTGGAC AGTTTTCTGA CGAGGTGGTT
          E  K  E   D  P  L   N  K  Y  T   F  W  E   V  N  L
1301    AGGAAAAGGA AGACCCATTG AACAAGTACA CCTTCTGGGA AGTCAACTTG
        TCCTTTTCCT TCTGGGTAAC TTGTTCATGT GGAAGACCCT TCAGTTGAAC
        K  E  K  F   S  A  D   L  D  Q   F  P  L  G   R  K  F
1351    AAGGAAAAGT TCTCTGCTGA CTTGGACCAA TTCCCATTGG GTAGAAAGTT
        TTCCTTTTCA AGAGACGACT GAACCTGGTT AAGGGTAACC CATCTTTCAA
         L  L  Q   S  G  L  K   A  K  P   R  L  K   R  S  A  P
1401    CTTGTTGCAA TCTGGTTTGA AGGCTAAGCC AAGATTGAAG AGATCTGCTC
        GAACAACGTT AGACCAAACT TCCGATTCGG TTCTAACTTC TCTAGACGAG
          T  T  R   A  P  S   T  K  R  K   K  V  K   K  *   (SEQ ID NO:2)
1451    CAACCACTAG AGCTCCATCC ACCAAGAGAA AGAAGGTCAA GAAGTAA (SEQ ID NO:1)
        GTTGGTGATC TCGAGGTAGG TGGTTCTCTT TCTTCCAGTT CTTCATT (SEQ ID NO:10
```

FIG.2C

Northern Blot of HPV 58 L1 wt and 58 L1 R transcripts.

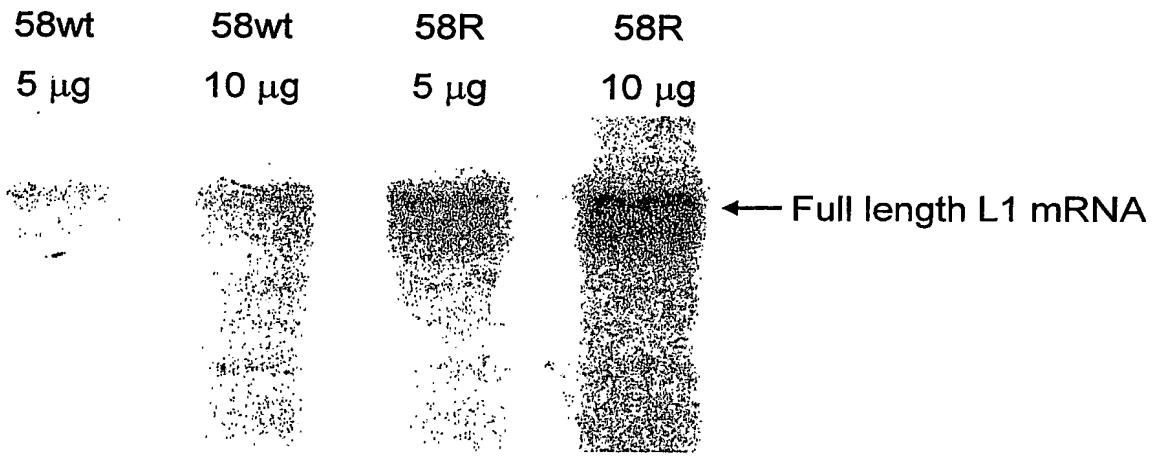

FIG.3

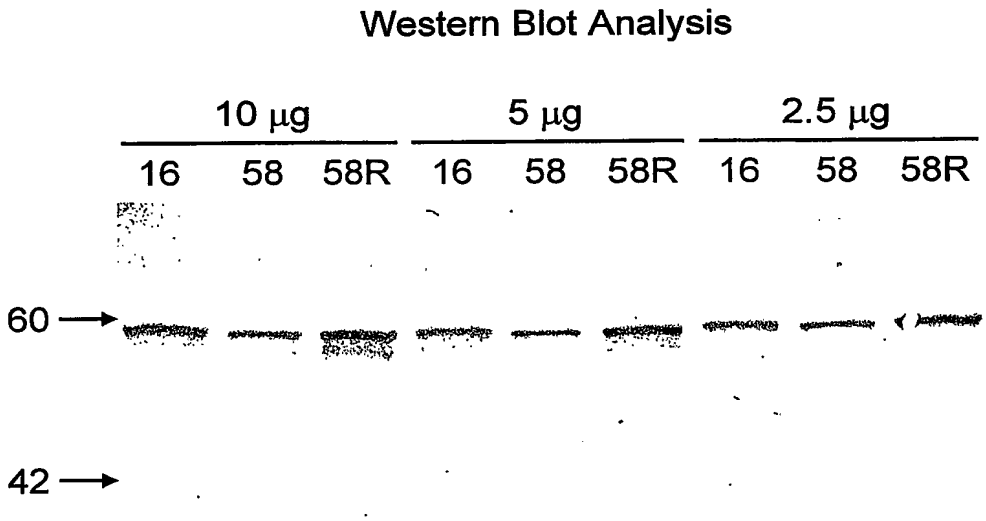
Western Blot Analysis
10 µg
5 µg
2.5 µg
16
58
58R
16
58
58R
16
58
58R
60
42

FIG.4

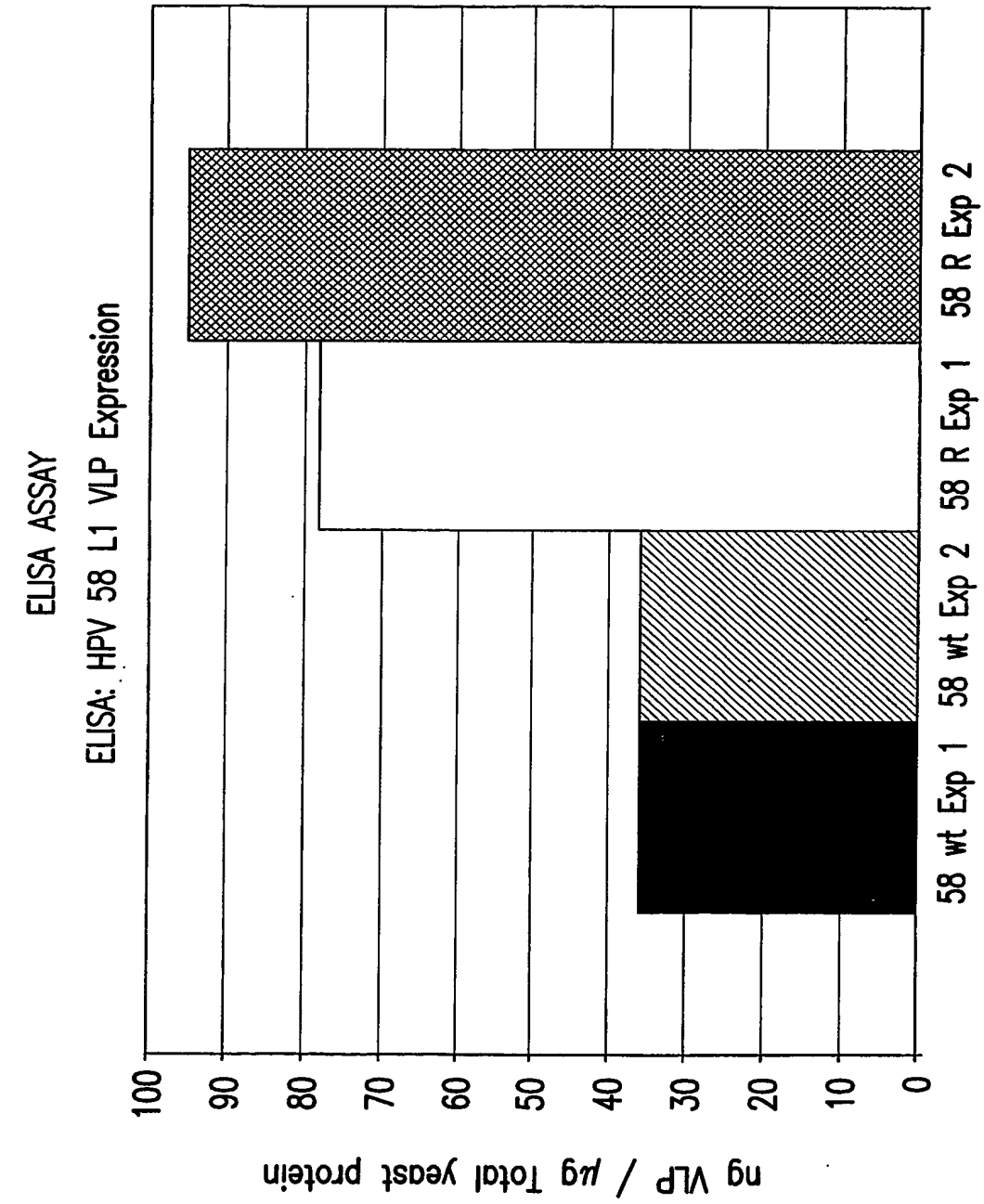
ELISA ASSAY
ELISA: HPV 58 L1 VLP Expression
ng VLP / µg Total yeast protein
100
90
80
70
60
50
40
30
20
10
0
58 wt Exp 1
58 wt Exp 2
58 R Exp 1
58 R Exp 2
HPV 58 L1 constructs

FIG.5

Transmission EM of VLPs Composed of HPV 58 L1 R Protein Molecules.

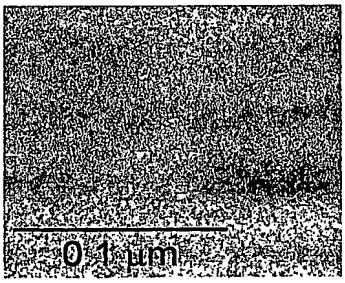

FIG.6